EVALUATION OF PRIMARY PHYTOCHEMICAL ANALYSIS AND DETECTION OF ANTIDIABETIC ACTIVITY OF *Punicagranatum* and *Carica papaya*

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ABSTRACT:

Punicagranatum and Carica papaya are two regularly consumed fruits whose photochemical makeup and anti-diabetic properties will be assessed in this study. In vitro experiments were used to measure the antidiabetic activity while several spectroscopic techniques were employed for the photochemical investigation. According to the study's findings, both fruits had a sizable anti-diabetic effect, albeit Punicagranatum had a more potent impact. Several bioactive substances, including flavonoids, phenols, and tannins, were detected by photochemical analysis, which may be the cause of the antidiabetic action that was reported. According to the research, Punicagranatum and Carica papaya ingestion may offer diabetics possible therapeutic advantages. The research study showed that *Punicagranatum* and *Carica papaya*exhibited various biological activities. We found that extract showed various primary metabolites presence and showed major biological activity like anti-diabetic activity, Based on the primary metabolites these extracts have anti-diabetic activity. Based on our in-vivo research studies, these extracts play a vital role in pharma to control the diabetics and act as herbal formulations in alternative medical fields.

Key words: Primary phytochemical ;*Punica* ; *Carica* ; antidiabetic activity ; pharm ; alternative medicine

INTRODUCTION:

The process of identifying, isolating, and quantifying the chemical substances found in plant extracts is known as phytochemical research. Phytochemicals, also referred to as secondary metabolites, are the chemical compounds that give plants their many biological characteristics, including their possible medicinal qualities.Peels from papaya and pomegranate are abundant in phytonutrients, including flavonoids, alkaloids, tannins, phenolic compounds, and other beneficial compounds. Numerous biochemical actions of these phytochemicals, including anti-diabetic effects, have been demonstrated for them.Analyzing a plant extracts or its separated components' capacity to reduce blood glucose levels in test subjects' animals or in vitro models is a crucial step in the detection of anti-diabetic activity. It can be done using a variety of techniques, such as glucose tolerance tests, insulin release assays, and glucose absorption assays.The papaya and pomegranate can be extracted using solvents like ethanol, methanol, or water to perform basic phytochemical analysis. The resulting extracts can then be put through various assays to determine and measure the various groups of phytochemicals present. These assays include the ferric reduction antioxidant power (FRAP) assay for antioxidant activity, the aluminum chloride assay for flavonoid content, and the Folin-Ciocalteu assay for total phenolic content.The phytochemical profile of the papaya and pomegranate extracts can then be determined, and the extracts can then be tested for their anti-diabetic action using the proper in vitro or in vivo methods. The findings of these studies can offer useful information on the possible use of these plant extracts as a homeopathic therapy for diabetes or as a source of new anti-diabetic compounds.

Diabetes has attracted attention due to its long-term health consequences, which include cardiovascular disease, lower limb amputations, and other disorders that interfere with life and raise mortality(Crawford , 2017). Patients with diabetes have a higher risk of infection because their immune systems are compromised. Secondary metabolites in natural products are a significant source of lead compounds for the improvement of pharmacological action due to their decreased toxicity and moderate side effects (Zhang, 2013).

Pomegranate is one of the attractive, colorful, and significant plant sources, and primarily its fruit include several therapeutic benefits (Prakash, 2014).The skins of the pomegranate fruit make up about 60% of its weight. Yet, the pomegranate peel may include antioxidants, a range of phytochemicals, and has antibacterial and antifungal qualities despite being considered an agricultural waste (Satheesh, 2012) .Pomegranate showed considerable antioxidant activity as a result of the presence of many active phytochemicals, such as catechins, polyphenols, anthocyanins, flavones, flavonoids in the peels, seeds, fruits, and kernels of pomegranate (Modaeinama, 2015).

There are several nations that are close to the equator where papaya is grown, including Malaysia, Brazil, South America, Australia, and Indonesia. Depending on where it grows, the plant Carica papaya L. is referred to by several various names, including kepaya, paw paw, and tapaya. This plant's fruit, seeds, roots, and leaves are only a few of the parts whose medicinal qualities are well acknowledged. As a result, it has been a part of conventional therapy for many illnesses .The Carica papaya L.'s most widely used proteolytic enzyme has been used to facilitate the breakdown and tenderization of meat. It is crucial to note that papain showed good potential as a medication (Yogiraj, 2014).Considering the nutrients present in its composition, it has been proven to significantly enhance the cardiovascular system and provide protection against cardiovascular conditions, heart attacks, and strokes (Wilson, 2002).

Research on pomegranate and papaya anti-diabetic properties and evaluation of primary phytochemical analysis is crucial for a number of reasons. Pomegranate and papaya extracts are known to include a variety of phytochemicals, such as polyphenols, flavonoids, and tannins. These compounds have been linked to a multitude of health benefits, including antidiabetic effect. The precise varieties and concentrations of phytochemicals in these extracts, as well as any possible antidiabetic properties, have not yet been adequately characterized. Second, innovative and efficient therapies are required due to the rising incidence of diabetes throughout the world. Therefore, discovering natural substances that have antidiabetic action, like those found in pomegranate and papaya extracts, may be a key to developing novel treatments for this illness.Thirdly, due to their widespread cultivation and use throughout most of the world, papaya and pomegranate extracts have the potential to be a plentiful and affordable source of antidiabetic chemicals. In conclusion, studies on the evaluation of primary phytochemical analysis and detection of antidiabetic activity of pomegranate and papaya extracts may offer crucial insights into the possible health advantages of these natural products and their prospective use as antidiabetic medicines.

We have various gaps in our knowledge of the potential health advantages of pomegranate and papaya fruits, which will be filled by the research on the topic "Evaluation of primary phytochemical analysis and detection of antidiabetic activity of pomegranate and papaya extracts": The phytochemical makeup of papaya and pomegranate peels and kernels is unknown. Despite the fact that pomegranates and papayas are widely known for their health advantages, less is known about the phytochemical makeup of their kernels and peels, which have a higher concentration of bioactive substances than their edible components.

Alternative diabetes treatments are required since the condition is common, affects millions of people globally, and has drawbacks with conventional therapies. As a result, there is a demand for alternative therapies that are secure, efficient, and cost-effective.Pomegranate and papaya may provide health advantages: Extracts from fruits like papaya and pomegranates have historically been utilized in folk medicine for their therapeutic benefits, which include antidiabetic action. There isn't enough scientific proof to back up these statements, though.

In order to examine the potential health advantages of pomegranate and papaya as natural antidiabetic agents, research on the subject will help close these knowledge and understanding gaps . This study aims in the comparative study of the primary phytochemical analysis and anti-diabetic activity of papaya and pomegranate.

MATERIAL AND METHODS

In present research, we have used several chemicals and strains to carry out the experiments and we purchased the chemicals, namely, Methanol, acetone, and distilled water, DPPH, Folin-Ciocalteu reagent, and RPMI-1640 medium, 10% FBS, 100 units/ from the vivo chemicals Pvt.Ltd.

The fruits of *Punicagranatum* and *Carica papaya* were collected from the local market, Chennai, and the specimens were identified.

Preparation of Plant Extracts

Ethanolic extract

The whole fruit of P. granatum and C. Papaya was dried in hot air oven at 40 -50℃ for a week . The dried plant material was powdered, and subjected to soxhlet extraction with 99% ethanol and chloroform for 24 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in the refrigerator for further use. The condensed extracts were used for preliminary screening of phytochemicals.

QUALITATIVE PHYTOCHEMICAL SCREENING

Table 1

Phytochemicals	P. granatum	C. Papaya
Triterpenoids	+	++
Alkaloid		
Dragendroff's reagent		+
Hager's reagent	-	+
Wagner's reagent	-	+
Flavonoids		
Shinoda test	+	++
Saponins		
Foam test	+	+

Table 2

Tannins/Phenolic substances		
Lead Acetate test	+	-
Ferric chloride test	+	-
Glycosides		
Alkali test	+	++
Steroids		
LibermannBuchard reaction	+	+
Carbohydrates		
Molisch test	+	++
Anthraquinones	-	+
Proteins	-	+
Amino acid		
Ninhydrin test	-	+
Fixed oil & Fats		
Soap test	-	-

Gums	-	+

'+' indicates presence and '–' indicates absence. ++ - Highly presence

QUALITATIVE TEST PROCEDURE

Test for alkaloids

About 1ml of the extract was stirred with a few drops of dilute hydrochloric acid and filtered. The filtrate was carefully tested with various alkaloidalreagents such as Dragondroff's reagent, Hager's reagent and Wagner's reagent.

Test for carbohydrates

The 1ml of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to a test for carbohydrates.

Molisch's test

The extract was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube.

Test for phytosterol

1ml of the extract was dissolved in a few drops of dry acetic acid, 3ml of acetic anhydride was added followed by a few drops of concentrated sulphuric acid. Appearance of bluish green shows the presence of phytosterol.

Test for Glycosides

Added glacial acetic acid, few drops of 5% ferric chloride and concentrated sulphuric acid to the 1 ml of extract. Observed reddish brown coloration at the junction of two layers and bluish green color in upper layer which indicates presence of glycosides

Test for fixed oils and fats

(a) About 1ml of the extract was separately pressed between two filter papers. Appearance of oil stains on the paper indicates the presence of fixed oil.

(b) Few drops of 0.5N alcoholic potassium hydroxide was added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated in a water bath for 1-2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Gums

Added 3ml of Dil. Hcl solution drops by drop to 1ml of test solution till red coloration occurs. Red coloration visualizes the presence of gums.

Test for triterpenoids

The plant extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet color shows the presence of triterpenoids.

Test for tannins and phenolic compounds

About 1ml of the extract was tested for the presence of phenolic compounds and tannins with

(a) Dilute ferric chloride solution (5%) - violet color
(b) 1% solution of gelatin with 10%NaCl - white precipitate
(c) 10% lead acetate solution - white precipitate

Test for Anthraquinone

To the 3ml of extract, dil. H_2SO_4 was added. The solution was then boiled and filtered. The

filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The upper layer did not turn pink showing the presence of Anthraquinone.

Test for Amino acids

Added a few drops of 40% NaOH and 10% of lead acetate to 5ml of test sample solution and boil the solution. Formation of black precipitate shows the presence of amino acids.

Test for proteins

The extract were dissolved in few ml of water and treated with

Biuret test: Equal volume of 5% solution of sodium hydroxide and 1% copper sulphate were added. Appearance of pink or purple colour indicates the presence of proteins and free amino acids.

Test for flavonoids

The extract were dissolved in alcohol, to that a piece of magnesium and followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

In Vitro antidiabetic activity

GLUCOSE UPTAKE ASSAY

L6 rat myogenic cells were cultured in hypotonic buffer (20 mMTris-HCl, pH 8, 1 mM EDTA, 0.2 mM EGTA, 50 mMNaF, 0.7 μg/ml pepstatin, 10 mM sodium orthovanadate, and 50 mMbenzamidine, 0.5 μg/ml leupeptin, 4 μg/ml aprotinin, and 2 mM**phenylmethylsulfonyl** fluoride). The cell suspension was centrifuged at 20,000 × g for 15 min and homogenate obtained. The supernatant was collected as the soluble fraction for further experiments.

L6 rat myogenic cells were seeded into a 96-well plate and let growing to confluence; then cells were fully differentiated in DMEM with 2% FBS for 5 days. Before tests, the medium was replaced by RPMI1640 (2 g/L glucose) supplemented with 0.2% BSA. The medium was removed after 2 h, and the same medium containing the extracts (20, 40, 60 and 80 mg/ml), metformin (10mg) the standard, and DMSO in absence or presence of insulin (1 μmol/L) was added to all wells including the blank. The glucose in the medium was determined by the glucose-oxidase method after 48 h treatment. The amount of glucose uptake by muscle cells was calculated by using the following formula:

Glucose uptake = Glucose conc of blank wells – Glucose conc of cells plated wells

Alpha-AMYLASE INHIBITION ASSAY

The fruit extracts with three different concentrations (20, 40, 60 and 80 mg/ml), were used for the study. A total of 500μl of plant extract and 500 μl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) containing α-amylase solution (0.5mg/ml) were incubated for 10 minutes at 25°C.After pre-incubation,500 μl of 1% starch solution in 0.02M sodium phosphate buffer (pH6.9 with 0.006 M sodium chloride) was added to each tube at 5s intervals. This reaction mixture was then incubated for 10 minutes at 25°C.1ml of DNSA colour reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540nm.

Statistical analysis:

Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference test. P values less

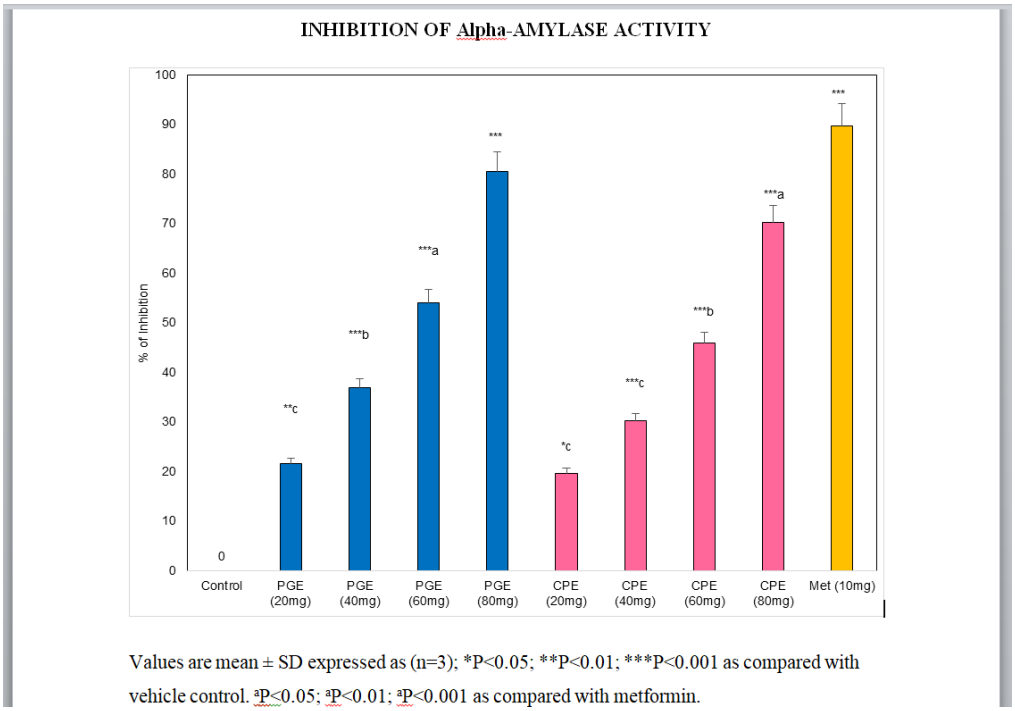
than 0.05 were considered significant. SPSS software was used for the statistical analysis (version 22.0).

GLUCOSE UPTAKE ASSAY

Table 3

Treatment	Conc(mg)	Glucose consumption (mg/100ml)	
		Absence of Insulin	**Presence of Insulin**
Control	0	1.62 ± 0.10	6.1±0.51
PGE	20	2.01± 0.11	5.19 ±0.4
	40	1.89 ±0.13	5.26±0.28
	60	1.74±0.56	5.38±0.21*
	80	1.45±0.50*	5.47±0.19*
CPE	20	2.99± 0.12*	4.13±0.14*
	40	2.01±0.78*	4.67±0.17*
	60	1.89 ±0.14	5.01±0.45
	80	1.67 ±0.65	5.11±0.36
Metformin	10	2.98± 0.12*	6.65±0.35*

Values are mean ± SD expressed as (n=3); *P<0.05 as compared with vehicle control.



Values are mean ± SD expressed as (n=3); *P<0.05; **P<0.01; ***P<0.001 as compared with vehicle control. ^{a}P<0.05; ^{a}P<0.01; ^{a}P<0.001 as compared with metformin.

Fig 1 Inhibition of Alpha Amylase activity

This study proved the presence of many active constituents in the ethanolic extract. The*Punicagranatum* extract and *Caricapapaya* extract exhibited good in vitro antidiabetic activity. The similar metformin standard efficacy was almost similar in*Punicagranatum* treatment compared with *Caricapapaya* treatment.

Extract preparation for phytochemical screening

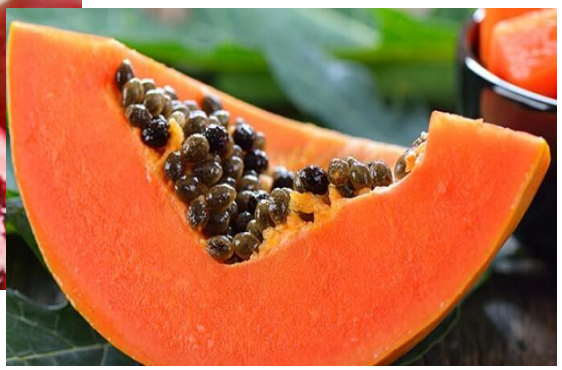


Fig 2: *Punicagranatum* Fig 3: *Carica papaya*

Fig 4: Extract of *PunicagranatumFig* 5: Extract of *Carica papaya*

In Vitro antidiabetic activity

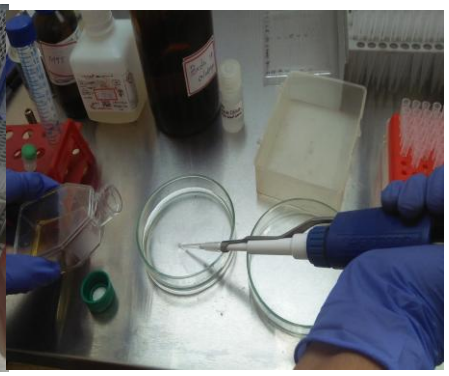
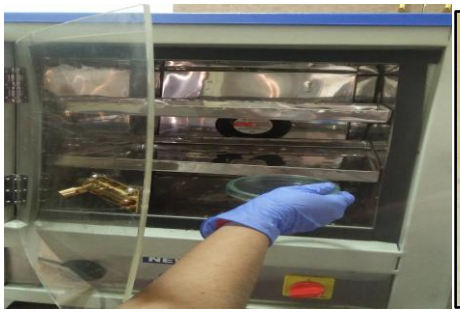


Fig 6 **Fig 7**



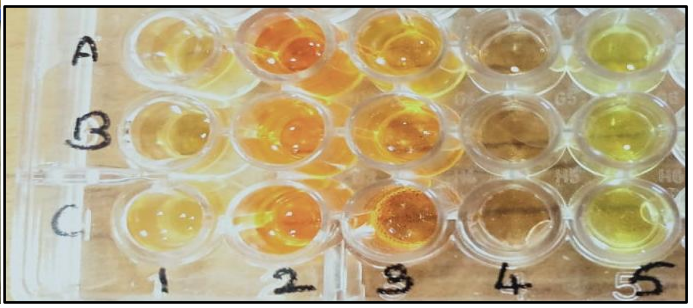


Fig 8 **Fig. 9**

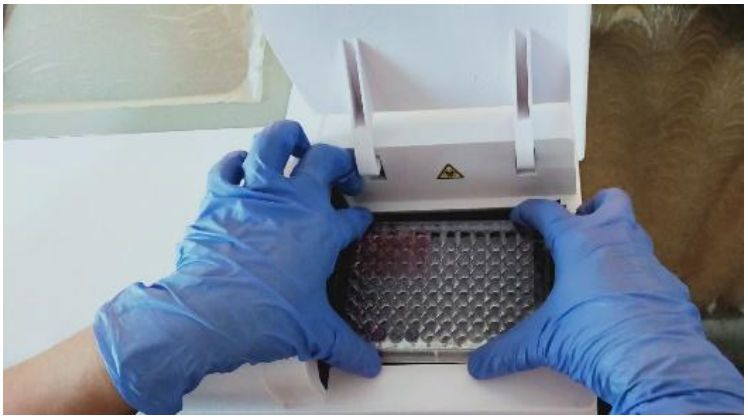


Fig 10

RESULT AND DISCUSSION

Findings give evidence for the effectiveness of Bergeniaciliate , Mimosa pudica, and Phyllanthusemblica as major inhibitors of α -amylase and α-glucosidase enzymes in the management of diabetes and support traditional uses of these plants as medicinal herbs(Basanta Kumar Sapkota , 2022). The chemical components of the papaya plant extract might prevent diabetes problems and provide an alternative to the current arsenal of anti-diabetic medications(TadesseBekeleTafesse, 2017). Numerous phytochemicals are present in pomegranate peel, which also possesses antimicrobial and antioxidant properties(Karthikeyan , 2019).Papaya seems to increase the blood glucose drop caused by glibenclamide, which appears to be interfering with the pancreatic insulin resistance (SudhakaraRao, 2022). Numerous laboratory-based studies have demonstrated that pomegranates are efficient in curing ailments by altering a wide range of biological processes(Arshad Husain Rahmani, 2017).Pomegranate extracts may be used as food preservatives without the adverse effects of artificial food additives thanks to their effective antibacterial action against verified food deterioration microorganisms(Mohamed TahaYassin, 2021). Antimicrobial properties in Carica papaya extract have been discovered to help in wound healing (Yew Rong Kong, 2021). Carica papaya seeds have demonstrated exceptional inhibitory effects against the diabetes-related glucosidase and amylase enzymes as well as oxidative stress, which may be the probable processes by which they reduce blood sugar levels(Reuben Agada, 2020). C papaya demonstrates therapeutic qualities, including reductions in drug-induced hepatotoxicity and nephrotoxicity, antibacterial, antimalarial, antiparasitic, anticancer, and anti-inflammatory activities, as well as the capacity to cure wounds (Lidiani F. Santana , 2019).

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